

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. 09/725,010
Applicant Paula R. Sundstrom
Filed 29 November 2000
TC/Art Unit 1635
Examiner J. Zara

Confirmation No. 8550

Docket No. 48544-00006
Customer No. 23767

DECLARATION OF PAULA R. SUNDSTROM, Ph.D.

Commissioner for Patents
Alexandria, VA 22313-1450

I, Paula R. Sundstrom, Ph.D., declare the following:

1. I am the inventor of the subject matter disclosed and claimed in referenced patent application, Serial No. 09/725,010.
2. I have read and I am familiar with the Office Action mailed 7 April 2004 pertaining to the referenced application. I understand that in the Office Action mailed 7 April 2004 the Examiner rejected claims 1, 4-7, 11, 19-21 and 27-32 under 35 U.S.C. § 112, first paragraph, for lack of written description and enablement.
3. I have also read and am familiar with the concurrently-filed Amendment and Reply under 37 C.F.R. § 1.111. Specifically, I have reviewed the claims as amended. Currently amended claim 1 reads as follows:

“A method comprising the step of:
inhibiting attachment of *C. albicans* to human tissue by interfering with
DNA binding proteins specific to UAS regions of the promoter of *HWP1* present
during germ tube formation in said *C. albicans*.”
4. The specification of the referenced application and, specifically, the experiments described therein as supported by the data presented herein, fully describe and enable, in my

opinion, one skilled in the art to practice the claimed inventions, including inhibiting attachment of *C. albicans* to human tissue by interfering with DNA binding proteins specific to UAS regions of the promoter of *HWP1* present during germ tube formation in *C. albicans*. See, e.g., pages 1-2, 6, 8, 10-11, 21-22, 24, 26, 53, 55, 62, 64-68, 72, and 75 of the specification. See also Figures 1-5 attached hereto. Accordingly, in my opinion, the present specification fully describes the inventions defined in the presently submitted amended claims and enables one skilled in the art to make and use those claimed inventions.

5. Specifically, experiments described in the referenced application and subsequently performed in my laboratory, at my direction, using Green Fluorescent Protein (“GFP”) as a reporter to assess the role of sequences upstream of the coding region show that specific UAS regions denoted “Morphogenic Response Region 1” (“MRR1”) and “Morphogenic Response Region 2” (“MRR2”) mediate expression of the *HWP1* gene in response to hypha-inducing conditions. See Figure 4 attached hereto. Deletion of DNA containing these regions leads to a 97% reduction in promoter activity as assessed by GFP expression relative to wild type. Without DNA sequences upstream of *HWP1* inclusive of these activating regions, the *HWP1* gene itself is not expressed.

6. Furthermore, the following evidences that *C. albicans* germ tubes, the predecessors of true hyphae, contain proteins that bind to these UAS regions of the *HWP1* promoter. Their presence in germ tubes and not yeast is important because expression of the *HWP1* gene occurs in germ tubes and true hyphae but not in yeast. Specific factors responsible for this morphogenetic regulation are predicted to be present in germ tubes and absent in yeasts. The presence of specific regions of activation, as shown in the specification and supported herein, coupled with evidence of such DNA binding proteins in germ tubes supports the presently claimed invention.

7. With regard to the promoter deletion experiments described in the referenced application, strains with maximal *HWP1* promoter activity, defined as 100%, were created by integration of the GFP reporter construct at the native *HWP1* locus where GFP expression was under the control of the entire region upstream of the transcription start site. Figure 5 shows the nucleotide

sequence of the *HWP1* promoter. Integration at an ectopic site, the *ENO1* locus, of wild type promoter constructs or constructs with external and internal deletions was performed to identify specific regions within the promoter that were important for GFP expression.

8. Figures 1A-1B present the results from our functional characterization of the *HWP1* promoter by analysis of external and internal deletion derivatives described in the specification and in Paragraph 7 above. Figure 1A shows fluorescence intensities of strains were determined in TCM199 after 3 h of growth at 37 °C. Values represent % of fluorescence of strains with the GFP reporter controlled by the entire *HWP1* promoter at its native locus (HB-12). The horizontal green bar indicates *HWP1* promoter DNA. The blue diamond indicates the position of the transcription start site. The vertical red bar just upstream of the blue diamond within the green bar signifies a TATAA element. Gaps connected by thin red lines indicate deleted regions.

9. Referring to Figure 1A, the fluorescence intensities of strains containing a construct 1902 bp upstream of the transcription start site were over 90% of the maximal promoter indicating that the sequences most responsible for promoter activation were found within this region (Construct -1902). Analysis of the other deletion derivatives (Figure 1A) showed that important activating regions were located between 1 and 2 kb upstream of the transcription start site in that deletion of 840 bp (-1063) resulted in a 97% reduction in fluorescence. A minor region of activation was found between -803 and -555 as shown by the reduction in fluorescence of 2.3% to 1.6%, the latter value being equivalent to the promoterless construct control. Thus, sequences between -1063 and -555 conferred a basal level of promoter activity.

10. Figure 1B is a graph showing fluorescence intensities of the various constructs relative to the wild type *HWP1* promoter. A bimodal pattern of activation within the 840 bp region between nucleotides -1902 and -1063 was shown by strains with external deletions. A distal activating region extended from -1902 to -1410 and accounted for over 60% of promoter activity in that deletion of 493 bp (-1410) reduced fluorescence to 38.8% of promoter activity at the native locus. The proximal segment spanning nucleotides, -1410 to -1063 accounted for approximately 36% (38.8 - 2.6) of promoter activity. Because most of the promoter activity in the distal segment was located within the first 246 nucleotides, as shown by the 45% decrease in

activity for construct -1657 compared to -1902, this region was denoted MRR1. Over half of the 45% decrease was attributable to the 125 nucleotides between -1782 and -1657 (72.3-45.7/45). An internal deletion of this region led to a 31% reduction in promoter activity (Construct L, Figure 1A).

11. A more proximal region of activation relative to the transcription start site was found spanning nucleotides -1410 to -1042. The function in activating expression was demonstrated by fusing this region to fragments with only basal promoter activity (external deletion -555 (Construct E, Figure 1A) and external deletion -803 (Construct C, Figure 1A)). Fluorescence intensities of 85 and 73%, for strains C and E, respectively relative to external deletion -1410 were found. Eleven percent of the fluorescence intensity of construct -1410 was present in strains with a fusion of segment -1288 to -1042 to external deletion -555 (Construct K2, Figure 1A). The 3' boundary of the proximal activating region was tested in constructs A and B, with 3' ends at -1130 or -1042, respectively, fused to external deletion -871 having basal promoter activity. Fluorescence intensity attributable to construct A was 30% of fragment B reflecting the importance of nucleotides between -1130 and -1042. The region between -1410 and -1042 was therefore denoted MRR2. Included within MRR2 was a repressing region between nucleotides -1410 and -1366 as shown by the increase in intensity from 38.8% to 58.7% for the two external deletions. This regulatory region within UAS MRR2 was termed MRR2a.

12. To show the importance of such UAS regions in controlling expression of Hwp1 during germ tube formation, an *hwp1/hwp1* null strain was transformed with a promoterless *HWPI* gene targeted to the *ENO1* locus created. Figure 2 illustrates the effect of the promoter deletion on Hwp1 expression. Indirect immunofluorescence assay using anti-Hwp1 antibodies was used to detect Hwp1 on germ tubes. Photographs were taken with 644 msec exposure times. SC5314 and the *hwp1/hwp1* null mutant which lacks the *HWPI* coding region are shown in A and B. Strains shown in C and D have the complete coding region of *HWPI* integrated at the *ENO1* locus; however, the strain shown in D lacks the *HWPI* promoter region.

13. As shown in Figure 2, Hwp1 was not present as deduced by anti-Hwp1 antibody in an indirect immunofluorescence assay. The control strain, which was constructed similarly except

that -1902 bp of *HWP1* upstream region was included, produced abundant Hwp1, showing that upstream sequences harboring UAS MRR1 and MRR2 are required for production of Hwp1 and its associated virulence attributes.

14. To search for DNA-binding proteins specific to the cis-activating regions (UAS regions), electrophoretic mobility shift (EMSA) experiments were performed. DNA fragments used in the analysis are shown in Figures 3A-3B. Results for MRR1 show the presence of DNA binding proteins that bind to the UAS MRR1 region of the promoter of *C. albicans* during germ tube formation.

15. Specifically, Figure 3A is a diagram representing regions used in EMSA analysis. Fragments with stars gave positive results in EMSA analyses, and therefore bind proteins in crude extracts of *C. albicans*. Stippled stars denote fragments shifted in both yeast and germ tube extracts whereas the fragment denoted by the solid star is positive with germ tube extracts only. Figure 3B depicts EMSA analysis using PCR fragments from distal regions of the *HWP1* promoter diagrammed above. Shifted band denoted by the solid star is found in germ tube extracts but not in yeast extracts. The shifted band is specific as it is competed by unlabeled specific DNA but not by non-specific DNA.

16. In light of the foregoing, in my opinion, the specification of the referenced application fully describes the presently claimed invention and enables a person skilled in the art to make and use the claimed invention. Indeed, the specification of the referenced application and the supporting data presented herein fully describe and enable one skilled in the art to make and use the claimed inventions, including inhibiting attachment of *C. albicans* to human tissue by interfering with DNA binding proteins specific to UAS regions of the promoter of *HWP1* present during germ tube formation in *C. albicans*.

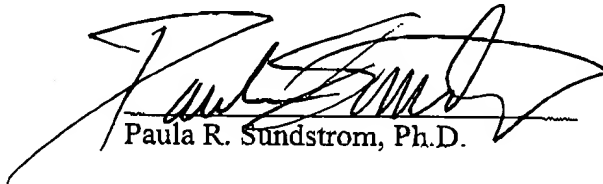
17. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

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imprisonment, or both, under section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

7 July 2004



Paula R. Sundstrom, Ph.D.